

of the most relevant determinants,⁴ but in our evaluation, the difference between the regions with or without reimbursement was marginal and not sufficient to explain the high rate of discontinuations. On the other hand, a recent report suggested that the frequency of control visits strongly affects the adherence,⁵ which seems to be plausible but could be not assessed in this context.

In conclusion, sales data provided by manufacturers show an alarming rate of SLIT discontinuation, which is approximately 90% at 3 years after prescription. The phenomenon is uniform and consistent, and its causes need to be investigated urgently, because adherence is of primary relevance for the efficacy of SLIT. A stricter and closer collaboration between clinicians and manufacturers could be a reasonable approach to study the problem and, possibly, solve it.

We thank Drs Paola Puccinelli, Franco Frati, and Cecile Hilaire (Stallergenes Italy) and Drs Enrica Loda and Massimo Milani (ALK-Abelló Italy) for kindly providing access to their marketing data.

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Disclosure of potential conflict of interest: G. Senna receives speakers' fees from Stallergenes Italy and ALK-Abelló Italy. G. Passalacqua receives speaker' fees from Stallergenes Italy and ALK-Abelló Italy. The rest of the authors have declared that they have no conflict of interest.

REFERENCES

1. Osterberg L, Blashke T. Adherence to medication. *N Engl J Med* 2005;353:487-97.
2. Senna G, Ridolo E, Calderon M, Lombardi C, Canonica GW, Passalacqua G. Evidence of adherence to allergen-specific immunotherapy. *Curr Opin Allergy Clin Immunol* 2009;9:544-8.
3. Lombardi C, Gani F, Landi M, Falagiani P, Bruno M, Canonica GW, et al. Quantitative assessment of the adherence to sublingual immunotherapy. *J Allergy Clin Immunol* 2004;113:1219-20.
4. Passalacqua G, Frati F, Puccinelli P, Scurati S, Incorvaia C, Canonica GW, et al. Adherence to sublingual immunotherapy: the allergists' viewpoint. *Allergy* 2009; 64:1796-7.
5. Vita D, Caminiti L, Ruggeri P, Pajno GB. Sublingual immunotherapy: adherence based on timing and monitoring control visits. *Allergy* 2010;65:668-9.

doi:10.1016/j.jaci.2010.06.045

Wheezing in preschool children is associated with increased levels of cytokines/chemokines in exhaled breath condensate

To the Editor:

Although asthma is a common disease in children, only a minority of young children with respiratory symptoms, such as wheeze, will develop persistent symptoms. Mostly, preschool wheeze is associated with viral respiratory tract infections. These children are usually symptom-free at the age of 6 years. Airway inflammation is a central feature in asthma. Whether and to what degree airway inflammation is already a characteristic in wheezing preschool children is unknown. The current tools to assess airway inflammation, such as lung biopsy and bronchoalveolar lavage, are too invasive for routine use in young children. There is an increasing interest in the noninvasive assessment of inflammatory markers in exhaled breath such as fractional exhaled nitric oxide (FeNO) and solutes in exhaled breath condensate (EBC).

The aim of our study was to assess whether FeNO levels, inflammatory markers in EBC, and airway resistance are associated with wheezing in preschool children.

This study included children from the Asthma DEtection and Monitoring (ADEM) study that started in 2006 in the Netherlands (registered at clinicaltrials.gov: NCT 00422747).¹ A total of 258 preschool children (age range, 1.9-4.5 years) were selected: 57 children who never wheezed, 78 children with 1 to 3 wheezing episodes, and 123 children who experienced more than 3 wheezing episodes during life (defined as recurrent wheeze), as assessed by the International Study of Asthma and Allergies in Childhood questionnaire.² Clinical characteristics are summarized in Table I. If applicable, inhaled corticosteroids administration was stopped at least 4 weeks before the measurements. The offline measurements of FeNO, the collection of EBC, and the measurements of airway resistance have been described in detail previously.¹ In short, FeNO was collected offline in a 500-mL inert balloon during tidal breathing and analyzed by using a nitric oxide monitoring system (NIOX; Aerocrine, Solna, Sweden). To collect EBC, a closed glass condenser system with a breath recirculation unit was used, which was developed at our institute.¹ This efficient condenser system enables the recirculation of exhaled breath that does not directly condense. As a result, a high amount of EBC can be collected in a short time. Acidity of EBC was analyzed directly after collection (Radiometer Benelux B.V., Zoetermeer, The Netherlands). Inflammatory markers in EBC (Interleukin [IL]-1 α , IL-2, IL-4, IL-5, IL-8, IL-10, IL-13, soluble intercellular adhesion molecule [sICAM], TNF- α , RANTES [Chemokine (C-C motif) ligand 5 (CCL5)], and eotaxin [CCL11]) were measured using multiplex immunoassay (Luminex Corp, Austin, Tex). Airway resistance before and after a short-acting β_2 -agonist (300 μ g salbutamol; Teva Pharma, Haarlem, The Netherlands) was measured by using the MicroRint device (Micro Medical Ltd, Rochester, United Kingdom).¹

Differences among the 3 groups regarding their clinical characteristics and main outcome measurements were evaluated by means of nonparametric tests (Kruskal-Wallis or Mann-Whitney) and the χ^2 test for categorical variables (SPSS Inc, Chicago, Ill). Adjustment for multiple testing was conducted via the false discovery rate. Differences among groups were considered significant when the corrected false discovery rate *P* value was <.05.

The results showed that 97% of the children (N=251) were able to produce sufficient EBC with a mean volume of 498 μ L (range, 60-1410 μ L). Sudden anxiety was the major reason for dropout of the remaining 7 children. Successful FeNO, baseline airway resistance, and postbronchodilator airway resistance measurements were achieved in, respectively, 99%, 96%, and 92% of the children.

All ILs (IL-1 α , IL-2, IL-4, IL-5, IL-8, IL-10, IL-13) and sICAM significantly differed between groups (*P* < .05, Kruskal-Wallis test). Results of the *post hoc* pairwise comparisons are shown in Fig 1 (A-C), in which the significant pairwise comparisons are marked with an asterisk (*P* < .05, Mann-Whitney test). Levels of all ILs and sICAM were higher in the group of children with >3 wheezing episodes than in the children who never wheezed. In addition, levels of IL-2, IL-4, and IL-10 were higher in children with minor symptoms (1-3 wheezing episodes) than in children who never wheezed. Both levels of IL-13 and sICAM were elevated in children with >3 wheezing episodes compared

TABLE I. Patient characteristics

Characteristic	>3 Wheezing episodes	1-3 Wheezing episodes	Never wheezed
Study population (no.)	123	78	57
Age (y), mean (range)	3.2 (2.0-4.4)	3.2 (1.9-4.3)	3.3 (2.2-4.5)
Male/female (no.)	66/57	45/33	28/29
Height (cm), mean (range)	97.0 (82.0-111.0)	98.2 (86.0-112.0)	98.3 (81.0-112.0)
Weight (kg), mean (range)	15.5 (9.1-22.1)	16.0 (11.7-23.0)	15.6 (10.5-20.4)
Atopy, no. (%)	27 (22)	18 (23)	13 (23)
Eczema, no. (%) [*]	51 (41)	27 (35)	13 (23)
Use of ICS, no. (%) [*]	30 (24)	7 (9)	0 (0)
Use of short-acting β_2 -agonist, no. (%) ^{**}	58 (47)	18 (23)	3 (5)
Use of long-acting β_2 -agonist, no. (%)	5 (4)	1 (1)	0 (0)

ICS, Inhaled corticosteroids.

^{*}*P* < .05: >3 vs 1-3 episodes, and >3 vs never wheezed.^{**}*P* < .05: >3 vs 1-3 episodes, >3 vs never wheezed, and 1-3 episodes vs never wheezed.

with the children with minor symptoms. No differences were found in levels of TNF- α , RANTES, and eotaxin and the pH of EBC between groups. Moreover, offline collected FeNO levels and airway resistance before and after salbutamol did not differ among the 3 groups. In a subanalysis, children with atopy were compared with children without atopy. Atopy was defined as a positive Phadiatop Infant test (Phadia, Uppsala, Sweden). There were no differences in FeNO levels, inflammatory markers in EBC (including the prototypical T_H2 cytokines IL-4, IL-5, and IL-13), and airway resistance between atopic and nonatopic children.

The high success rate of EBC collection in our study is the result of various factors. At first, we used a closed-glass condenser that enables recirculation of exhaled breath that does not directly condense. In this way, noncondensed air is not lost, in contrast with condensers with an open end. The recirculation increases the amount of collected EBC 2-fold, and there is no indication that it influences the levels of markers, because measurements before and after breath recirculation did not significantly differ.³ The efficient system enables sufficient EBC collection in less time (10 minutes is enough), which is an important success factor in this young age group. Second, EBC markers were measured by using multiplex immunoassay (Luminex technology). This technology is highly sensitive and is able to measure different cytokines accurately in a small sample volume.⁴ Finally, we increased feasibility by facilitating tools to make children feel at ease during the EBC collection.

The increased levels of the various exhaled inflammatory markers in condensate point to an ongoing inflammatory response in children with recurrent wheeze compared with nonwheezing controls. The elevated markers in recurrent wheezers may reflect different aspects of airway inflammation. They include T_H2 cytokines (IL-4, IL-5, IL-13), a proinflammatory cytokine (IL-1 α), a T_H1 cytokine (IL-2), a chemokine (IL-8), an anti-inflammatory cytokine (IL-10), and an adhesion molecule (sICAM). These markers play an important role in the inflammatory response in asthma. The elevated levels of different types of cytokines fit into the concept that in wheezing preschool children, more than 1 immune mechanism is involved, leading to a chain of inflammatory events.⁵

The etiology of preschool wheezing is heterogeneous. Some children may wheeze because of a viral infection; in others, allergen provocation may trigger their symptoms. The type of inflammation differs depending on the underlying

pathophysiology. Viral wheeze is the most common phenotype of wheezing in preschool children. There is increasing evidence that during a viral-triggered wheezing attack, neutrophilic inflammation plays a central role.^{6,7} In contrast, in children with atopic asthma, inflammation is predominately mediated by eosinophils.⁸ Some of our results point to a neutrophilic inflammation in the children with recurrent wheezing. For this group, we found elevated levels of IL-8, a neutrophil chemoattractant. By contrast, we did not find elevated levels of RANTES and eotaxin, 2 powerful chemoattractants for eosinophils, in wheezing children. Similarly, no group difference in offline collected FeNO levels, known to reflect eosinophilic inflammation, was observed. However, we did find elevated levels of IL-5 in children with recurrent wheeze, a marker involved in the activation of eosinophils. Therefore, our results strengthen the current opinion that multiple mechanisms lead to episodes of wheeze at preschool age.⁵

No differences in FeNO levels and EBC markers in children with atopy compared with children without atopy were found. This may be a result of the low prevalence of atopy to inhaled allergens in this age group, resulting in small numbers per group.

In previous studies, bronchoscopy and bronchoalveolar lavage were performed to examine airway inflammation in preschool wheezers.^{9,10} An advantage of the EBC technique over bronchoscopy and bronchoalveolar lavage is that EBC collection is a safe, noninvasive technique and can be easily performed in nonsedated preschool children. However, low detection limits and possible influences of expiratory flow, temperature of the condenser tube, and dilution factor on EBC collection remain challenging issues of this technique that need to be addressed in further investigations. Future pediatric studies comparing markers in EBC with markers in bronchoalveolar lavage and induced sputum are important to increase our knowledge about the pathophysiology of preschool wheezing.

In conclusion, we demonstrated that EBC collection, offline FeNO, and airway resistance measurements are feasible in preschool children. The elevated EBC markers indicated increased airway inflammation in preschool children with recurrent wheezing. The analysis of EBC is helpful as a noninvasive tool for the early detection of airway inflammation in preschool children with respiratory symptoms. In the ADEM study, we will follow the children until the age of 6 years. At that age, we will be able to distinguish different wheezing phenotypes. Whether markers in

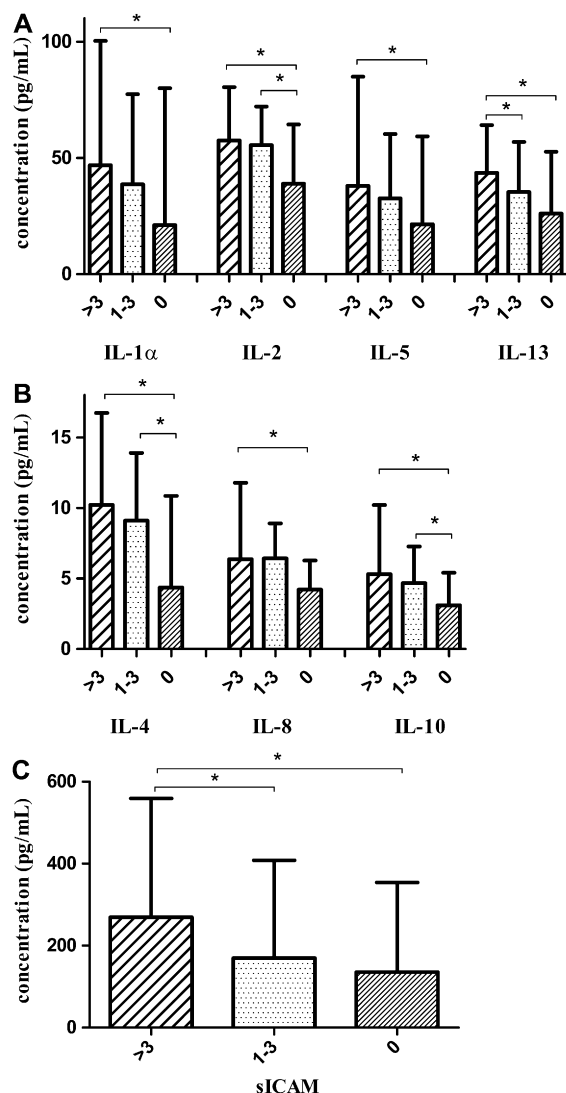


FIG 1. A-C, Markers in EBC of children with >3 wheezing episodes (coarsely-striped bar), 1 to 3 wheezing episodes (spotted bar), and no wheezing (finely-striped bar). *Significant pairwise comparisons ($P < .05$). Markers are grouped based on their range in concentration.

EBC, FeNO, and MicroRint measurements are useful to distinguish between wheezing phenotypes, will be determined during this follow-up.

We thank our research nurse, Karen Groot, and master's degree student in health sciences, Annedien Nijhuis, for their outstanding assistance during the collection of EBC and the lung function measurements.

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Supported by unrestricted grants from the Dutch Asthma Foundation (NAF 3.4.05.033), Stichting Astma Bestrijding (SAB 2006/018), and Maastricht University Medical Center (PF 294).

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

REFERENCES

- van de Kant KD, Klaassen EM, Jobsis Q, Nijhuis AJ, van Schayck OC, Dompeling E. Early diagnosis of asthma in young children by using non-invasive biomarkers of airway inflammation and early lung function measurements: study protocol of a case-control study. *BMC Public Health* 2009;9:210.
- Worldwide variation in prevalence of symptoms of asthma, allergic rhinconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. *Lancet* 1998;351:1225-32.
- Rosias PP, Robroeks CM, van de Kant KD, Rijkers GT, Zimmermann LJ, van Schayck CP, et al. Feasibility of a new method to collect exhaled breath condensate in pre-school children. *Pediatr Allergy Immunol* 2010;21:e235-44.
- de Jager W, te Velthuis H, Prakken BJ, Kuis W, Rijkers GT. Simultaneous detection of 15 human cytokines in a single sample of stimulated peripheral blood mononuclear cells. *Clin Diagn Lab Immunol* 2003;10:133-9.
- Martinez FD, Godfrey S. Immunology of wheezing disorders in infants and preschool children. New York: Martin Dunitz; 2003.
- McDougall CM, Helms PJ. Neutrophil airway inflammation in childhood asthma. *Thorax* 2006;61:739-41.
- Marguet C, Jouen-Boedes F, Dean TP, Warner JO. Bronchoalveolar cell profiles in children with asthma, infantile wheeze, chronic cough, or cystic fibrosis. *Am J Respir Crit Care Med* 1999;159:1533-40.
- Drewe AC, Pizzichini MM, Pizzichini E, Pereira MU, Pitrez PM, Jones MH, et al. Neutrophilic airway inflammation is a main feature of induced sputum in non-atopic asthmatic children. *Allergy* 2009;64:1597-601.
- Le Bourgeois M, Goncalves M, Le Clairche L, Benoist MR, Fournet JC, Scheinmann P, et al. Bronchoalveolar cells in children < 3 years old with severe recurrent wheezing. *Chest* 2002;122:791-7.
- McNamara PS, Flanagan BF, Baldwin LM, Newland P, Hart CA, Smyth RL. Interleukin 9 production in the lungs of infants with severe respiratory syncytial virus bronchiolitis. *Lancet* 2004;363:1031-7.

doi:10.1016/j.jaci.2010.07.013

Impaired T_H2 response in patients with Vav1-deficient common variable immunodeficiency with T-cell defects

To the Editor:

Common variable immunodeficiency (CVID) is a primary immune disorder including a heterogeneous group of diseases with the unifying feature of hypogammaglobulinemia.¹ Defects not only in B cells, which are directly responsible for antibody production, but also in other immune cells involved in the generation of an effective humoral response, including antigen-presenting cells and T_H cells, represent the molecular basis of CVID.²

T_H cells orchestrate the host immune response and can be divided into different subsets based on their pattern of cytokine secretion. T_H1 cells produce IFN-γ and TNF-β, which elicit macrophage activation and cell-mediated immunity, whereas T_H2 cells produce IL-4 and IL-13, which promote B-cell maturation and antibody production. The differentiation of T_H1 and T_H2 subsets is finely regulated by specific transcription factors, cytokines, and costimulatory signals. Among the lineage-specific transcription factors, T-box transcription factor (T-bet) plays a central role in T_H1 cell development, whereas GATA-3 and c-Maf drive T_H2 cell differentiation.³⁻⁵

We have previously identified a subset of patients with CVID with impaired T-cell function characterized by defective